Biological Activity of *Merremia emarginata* Crude Extracts in Different Solvents

A.V. Babu, R.S.C. Rao, K.G. Kumar, B.H. Babu and P.V.V. Satyanarayana
Department of Chemistry, Acharya Nagarjuna University,
Nagarjuna Nagar-522 510, A.P., India

**Abstract:** The plant *Merremia emarginata* (Burm. f.) Hallier f., belongs to Convolvulaceae family. In traditional medicinal system, different parts of *M. emarginata* have been mentioned to be therapeutically used as deobstruent, diuretic, for cough, headache, neuralgia and rheumatism. In the present study, biological activities of different solvent extracts isolated from *M. emarginata* were tested. Hexane (IA), ethyl acetate (IB), methanol (IC) and aqueous methanol (25%) (ID) extracts of *M. emarginata* were examined. Antioxidant properties of the extracts were studied by DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity method and superoxide radical scavenging activity method. Methanol extract exhibited better antioxidant activity than other extracts with IC$_{50}$ of 8.59 μg mL$^{-1}$ in DPPH radical scavenging method. Methanol and hexane extracts exhibited α-amylase inhibitory activity with IC$_{50}$ of 104.5 and 133.4 μg mL$^{-1}$, respectively. Ethyl acetate extract showed cytotoxicity with ED$_{50}$ of 3.49 μg mL$^{-1}$ in brine shrimp lethality assay. The present study revealed that the extracts IB and IC of *M. emarginata* were found to be showed promising biological activities. Methanol extract of this plant might be use full for antioxidant and antiobesity activities with minimal toxicity.

**Key words:** *Merremia emarginata*, antioxidant, α-amylase inhibitory activity

**INTRODUCTION**

Natural products produced by plants, microorganisms, insects and animals have been isolated as biologically active pharmacophores (Gordon and David, 2005; Cragg and Newnun, 2007; Wang *et al.*, 2006). Approximately one-third of the top-selling drugs in the world are natural products or their derivatives often with ethnopharmacological background (Kingston, 2009). The advantage of natural products for random screening is the structural diversity provided by natural products, which is greater than provided by most available combinatorial approaches based on heterocyclic compounds (Harvey, 1999). Cox (1994) suggested that the ethno-directed sampling is most likely to succeed in identifying drugs used in the treatment of cancer, inflammatory and dermatological complaints. According to Chatterjee *et al.* (2006) tribal healers in most of the countries, the ethnomedical treatment is frequently used to treat cuts wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbs. India possesses rich floristic wealth and diversified genetic resources of medicinal plants (Arora *et al.*, 2003).

**Corresponding Author:** B. Hari Babu, Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, A.P., India
The use of the plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds (Alluri et al., 2006).

Antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative stress related diseases such as autoimmune (Willet, 1994) cardiovascular (Vinson et al., 1995) and neurovascular diseases (Aggarwal and Harikumar, 2008). Recently, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have higher antioxidant activities than those of conventional vitamins C, E and β-carotene (Hafidh et al., 2009). Cytotoxicity screening models like brine shrimp lethality assay are the preliminary methods for selection of active plant extracts against cancer (Al-Fatimi et al., 2007). The management of diabetes by chemical drugs without any side effects is still a challenge to the medical system. Many efforts have been made to identify new antidiabetic agents from different sources, especially medicinal plants because of their effectiveness, fewer side effects and relatively low cost (Bhandari et al., 2008). The stable blood glucose level is important for diabetic patients, because it prevents the hyperglycaemia and the complications associated with diabetes. Carbohydrate hydrolyzing enzyme like α-amylase inhibitors are one of the essential drugs for managing type II diabetes (Nickavar et al., 2008).

The plant *Merremia emarginata* (Burm. f.) Hallier f., belongs to Convolvulaceae family. In Sanskrit, it is called as Mooshikakarnee (Satyavati et al., 1987). In India, it is mainly found in Chennai and in some places of Andhra Pradesh (Pollaiah et al., 2000). The plant was therapeutically used as deobstruent, diuretic, for cough, headache, neuralgia and rheumatism (Chatterjee and Prakash, 1995). The importance of *M. emarginata* as a biologically potent plant species was proposed the study in vitro antioxidant, α-amylase inhibition and cytotoxicity activities of different solvent extracts of *M. emarginata* is reported.

**MATERIALS AND METHODS**

**Plant Material**

The plant material (whole plant except flowers) was collected from the Botanical Garden of Acharya Nagarjuna University, Nagarjuna Nagar and authenticated by the Department of Botany.

**Preparation of Extracts**

The plant material was collected and air dried. Finely powdered plant material (1 kg) was extracted twice with hexane (5 L) and then the spent was continuously extracted with ethyl acetate, methanol and aqueous methanol (accordingly). The solvent extracts were collected and dried by using rotary vacuum evaporator. The dried weights of the crude extracts are as follows hexane (IA): 12.3 g, ethyl acetate (IB): 16.65 g, methanol (IC): 30 g and aqueous methanol (ID): 15 g.

**Antioxidant Activity**

**DPPH Radical Scavenging Activity Method**

The DPPH (1,1-Diphenyl-2-Picrylhydrozyl) radical scavenging activity was determined according to the method of Szabo et al., (2007), the Optical Density (OD) of colored methanolic solution of the DPPH free radicals was measured at 517 nm. Percent of inhibition was calculated by comparing absorbance of crude extract with that of control. The radical scavenging activity of the crude extracts was expressed as the percent of inhibition and the IC₅₀ values were obtained from the plot drawn concentration (μg) verses % of inhibition.
Superoxide Radical Scavenging Activity Method

Superoxide radicals were generated in vitro by non enzymatic system and determined spectrophotometrically (560 nm) by Nitro Blue Tetrazolium (NBT) photo reduction method (McCord and Fridovich, 1969). The assay mixture consist of 6.5 mM EDTA containing 3 μg of NaCN, 2 μM of riboflavin, 50 μM of NBT, crude extract and 67 mM of phosphate buffer (pH 7.8) in a final volume of 3 mL. The optical density at 560 nm was measured before and after 15 min illumination. The superoxide radical scavenging activity of the crude extracts was expressed in IC₅₀ values.

Alpha-Amylase Inhibitory Activity

The α-amylase activity was measured using the dinitrosalicylic acid (DNS) method developed by Bernfeld (1955), improved by Jamieson et al. (1969) and adopted for testing α-amylase inhibitory potential (Da silva et al., 2004) using 1% soluble starch as substrate. The test substance was pre-incubated with amylase (100 μl) at room temperature for 20 min prior to the addition of 100 μl of the substrate solution followed by incubation at 37°C for 10 min. The reactions were stopped by the addition of 200 μl of DNS reagent followed by color development by placing the tubes in boiling water for 5 min and then added 3.6 ml of distilled water. Acarbose was used as positive control. The absorbance was read at 470 nm and experiments were carried out in duplicates.

Brine Shrimp Lethality Bioassay

Brine shrimp (Artemia salina) nauplii were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial sea water of 38 g L⁻¹ of sea salt and adjusted to pH 8.5 using 1 N of NaOH and kept under constant aeration for 48 h. After hatching, 10 nauplii were drawn through a pipette and placed in each vial containing 4.5 mL brine solution and added various concentrations of crude extracts (0-300 μg mL⁻¹) and the final volume was made up to 5 mL using brine solution and maintained 37°C for 24 h under the light of incandescent lamps (McLaughlin et al., 1993, 1998). Assays were carried out in duplicates. The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. Podophyllotoxin was used as a positive control.

RESULTS

The crude extracts IA, IB, IC and ID were studied for antioxidant, α-amylase inhibitory and cytotoxic activities. The antioxidant activity was measured in two biological methods namely DPPH and superoxide radical scavenging activity method. The results of the two methods were incorporated in Table 1. In DPPH method, the IC crude extract showed better anti-oxidant activity (8.59 μg mL⁻¹) followed by IB (21.5 μg mL⁻¹), ID (31.2 μg mL⁻¹) and IA (56.2 μg mL⁻¹). In superoxide radical scavenging activity method, the extract IB exhibited

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>DPPH radical scavenging method</th>
<th>Superoxide radical scavenging method</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>36.20</td>
<td>87.00</td>
</tr>
<tr>
<td>IB</td>
<td>21.50</td>
<td>45.10</td>
</tr>
<tr>
<td>IC</td>
<td>8.59</td>
<td>54.30</td>
</tr>
<tr>
<td>ID</td>
<td>31.20</td>
<td>89.40</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>3.35</td>
<td>150.00</td>
</tr>
</tbody>
</table>

IA, IB, IC and ID are crude extracts of hexane, ethyl acetate, methanol and aqueous methanol, respectively.
**Table 2: Alpha amylase inhibitory activity of the crude extracts**

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>IC₅₀ (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>133.4</td>
</tr>
<tr>
<td>IB</td>
<td>421.8</td>
</tr>
<tr>
<td>IC</td>
<td>104.5</td>
</tr>
<tr>
<td>ID</td>
<td>218.0</td>
</tr>
<tr>
<td>Acarbose</td>
<td>10.9</td>
</tr>
</tbody>
</table>

IA, IB, IC and ID are crude extracts of hexane, ethyl acetate, methanol and aqueous methanol, respectively.

**Table 3: Brine shrimp lethality test for the crude extracts**

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>ED₅₀ (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>74.15</td>
</tr>
<tr>
<td>IB</td>
<td>34.29</td>
</tr>
<tr>
<td>IC</td>
<td>237.41</td>
</tr>
<tr>
<td>ID</td>
<td>145.40</td>
</tr>
<tr>
<td>Standard (Podophyllotoxin)</td>
<td>2.36</td>
</tr>
</tbody>
</table>

IA, IB, IC and ID are crude extracts of hexane, ethyl acetate, methanol and aqueous methanol, respectively.

IC₅₀ at 45.1 µg mL⁻¹ followed by IC (54.3 µg mL⁻¹), IA (87.09 µg mL⁻¹) and ID (89.4 µg mL⁻¹).

The *in vitro* α-amylase inhibitory activity (Table 2) results revealed that the extract IC showed IC₅₀ at 104.5 µg mL⁻¹ followed by IA. The brine shrimp results were incorporated in Table 3, the extract IB showed good cytotoxicity against brine shrimp larvae followed by IA. The ED₅₀ value of the standard podophyllotoxin is 2.36 µg mL⁻¹.

**DISCUSSION**

We know that variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases (Fabricant and Farnsworth, 2001). Oxygen is easily activated by Ultra Violet (UV) radiation and heat from the sunlight to produce toxic Reactive Oxygen Species (ROS). These ROS are highly reactive because they can interact with a number of cellular molecules and metabolites there by leading to a number of destructive processes causing cellular damage (Hafidh et al., 2009). Researchers have been reported that convolvulaceae member like *Ipomoea aquatica* and *L. batatas* L. was showed potent antioxidant properties in their leaves and shoots. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc. (Aher et al., 2009). The chloroform and ethyl acetate extracts of the aerial part of *Maranta quadrifolia* have been reported profound antibacterial, cytotoxic and antioxidant effects (Ripa et al., 2009). The free radical scavenging activity of the 70% aqueous methanol and ethyl acetate extracts of *Annona senegalensis* leaves is due to their constituents of polyphenols (Potchau et al., 2008). In the present study, methanol and ethyl acetate extracts from *M. emarginata* were found to be showed better antioxidant activities than other extracts.

Alpha-amylase inhibitors are drug-design targets for the development of compounds for treatment of diabetes, obesity and hyperlipaemia (Franco et al., 2000). Nickavar and Mosazadeh (2009) reported that three *Morus* species extracts have been exhibited *in vitro* α-amylase inhibitory activity with IC₅₀ of 12 mg to 18 mg mL⁻¹. Methanol and hexane extracts of *M. emarginata* were found to be showed *in vitro* α-amylase inhibition with IC₅₀ of 104.5 and 133.4 µg mL⁻¹, respectively. These results revealed that the IC extract has superior α-amylase inhibition activity than other substrates.
Brine shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection plant extract toxicity (McLauglin et al., 1991). The use of the brine shrimp assay to screen plant extracts has been used successfully to biomonitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent compounds from plant extracts (Alluri et al., 2006). Moshi et al. (2009) reported that the extracts from different plants exhibited toxicity against brine shrimp larvae with LC₅₀ values ranging from 15.35-37.4 μg mL⁻¹, ethyl acetate and dichloromethane extracts have been showed more cytotoxicity. In the present study, ethyl acetate extract from M. emarginata was showed better cytotoxicity effect than other extracts. Only few reports are available on biological activities of Merremia species. Austin (2007) reviewed the uses of Merremia dissecta (Convolvulaceae), the plant has been used for the treatment of inflammations, itching, snake bites and as intoxicant. The extracts of M. emarginata, belongs to the same family have not been reported previously for biological properties.

CONCLUSION

In traditional medicinal system, different parts of M. emarginata have been mentioned to be therapeutically used as deobstructant, diuretic, for cough, headache, neuralgia and rheumatism. The present study revealed that the ethyl acetate (IB) and methanol extracts (IC) of M. emarginata were found to be showed promising biological activities. Methanol extract of this plant might be use full for antioxidant and antidiabetic activities with minimal toxicity.

ACKNOWLEDGMENT

The authors wish to thank Dr. G. Trimurthulu, Vice-President, Laila Impex R and D Centre, Vijayawada, for his help in biological activity studies.

REFERENCES


